

## Amendments to the Specification

Please amend page 2, lines 23-27:

The main disadvantage of vaccines derived from virulent pathogens is, that they must be carefully and sufficiently attenuated in order to be safe.

This requirement can relatively easy be fulfilled for highly virulent microorganisms, because they can in most cases be highly attenuated while ~~[[remaining]]~~ retaining their vaccinating (immunity-inducing) capacities.

Please amend page 3, lines 23-37:

*Ornithobacterium rhinotracheale* is a relatively new bacterium causing a disease known for about a decade now, and found frequently in ~~[[i.a.]]~~ inter alia, chickens and turkeys. Clinical signs in chickens are e.g. airsacculitis or coughing, pneumonic lungs or pleuritis. In turkey flocks in several parts of the world, a comparable infection of the respiratory tract is found. Mortality in flocks suffering from the disease can be as high as 5%. The first clinical signs are comparable to infection in chicken: sneezing and nasal discharge. In some animals clinical signs of acute infection are seen. Examination of sacrificed animals shows edema of the lungs, fibrinopurulent pneumonia and often serofibrinous pericarditis and serofibrinous infection of the airsacs. *Ornithobacterium rhinotracheale* is extensively described in European Patent EP0.625.190. Identification, serotyping and experimental infection in turkeys and chickens have been described e.g. by van Empel, P.C.M. et al., in Journ. of Clin. Microbiol. 35: 418-421 (1997), by van Empel, P.C.M. et al., in Avian Diseases 40: 858-864 (1996) and by van Empel, P.C.M. et al., in Avian Pathology 28:217-227 (1999). A review on *Ornithobacterium rhinotracheale* has been published in Avian Pathology 28: 217-227 (1999) by van Empel, P.C.M. and Hafez, H.M.

Please amend page 12, lines 29-31:

### Oligonucleotides

All oligonucleotides were obtained from Life Technologies <sup>TM</sup> Gibco BRL (Paisley, UK) and are indicated in Table 1. Their location in the sequence is shown in ~~[[figure]]~~ Figures 2A and B.

Please amend page 13, lines 10-12 (Table 1.):

Table 1. Oligonucleotides (primers) used for preparation of insertion-deletion constructs.

Primer Name	Sequence (5' → 3')	Features
<i>PurD</i> -F13	CTTAAGCTTGGATCCTTGTGGCGTGGCTTTAG [SEQ ID NO.:1]	<i>HindIII</i> site (underlined)
<i>PurD</i> -OE-R	CTTCTAGCGTAGCGCCAGATCTCATTTGTTCGGT TCCAGCGTTTCC [SEQ ID NO.:8]	<i>BglII</i> site (underlined) and overlap with <i>PurD</i> -OE-F (italics)
<i>PurD</i> -OE-F	GAGATCTGGCGCTACGCTAGAAGAAGCC [SEQ ID NO.: 7]	<i>BglII</i> site (underlined) and overlap with <i>PurD</i> -OE-R (italics)
<i>PurD</i> -R8	CTTAAGCTTTCAGTGGAGCGGCAGATACAGAG_ [SEQ ID NO.:6]	<i>HindIII</i> site (underlined)
<i>RecA</i> -F6	CTTAAGCTTGGAGCGTGTAGTGCTCGCCATCG [SEQ ID NO.: 5]	<i>HindIII</i> site (underlined)
<i>recA</i> -OE-R	ACCGCACGCACGAGATCTCGGGCTTTGTGCCCC ATCATCATCAC [SEQ ID NO.: 4]	<i>BglII</i> site (underlined) and overlap with <i>recA</i> -OE-F (italics)
<i>recA</i> -OE-F	CGAGATCTCGTGCCTGCGGTATTGAAAG [SEQ ID NO.: 3]	<i>BglII</i> site (underlined) and overlap with <i>recA</i> -OE-R (italics)
<i>RecA</i> -R5	CTTAAGCTTCCCAGCCAATTCGGCTCGTTTCAC_ [SEQ ID NO.: 2]	<i>HindIII</i> site (underlined)

Please amend page 14, lines 23-28:

## RESULTS

### Sequencing *recA* and *purD*.

To obtain the sequence of the *purD* and *recA* genes of *OR*, degenerate primers were developed based on conserved regions of the genes derived from closely related bacteria. By means of genome walking, the flanking regions were determined. The complete sequences and the relevant features are shown in [[figure]] **Figure 2A** for *purD* and [[figure]] **Figure 2B** for *recA*.

Please amend page 16, lines 17-29:

The figures of the respiratory lesion score (see column resp. score in [[table]] **Table 2**) are to be interpreted as follows: the maximal possible lesion score is taken as 100% score. Non-vaccinated animals show a lesion score of 51%, and “vaccinated” animals have a lesion score of 46% (*RecA*) or 35% (*PurD*). This means that “vaccination” with *RecA* or *PurD* gives a level of protection of 8% (100%-(46\*100%/51)) or 30% respectively.

A protective immune response, as defined above, is an immune response that gives, after challenge, a (statistically significant) decrease in respiratory tract lesion score of equal or more than 50% compared to non-vaccinated animals.

It follows from **Table 2** that the respiratory lesion score of the groups that received vaccination with a mutant of *Ornithobacterium rhinotracheale* does not significantly differ from the respiratory lesion score found with the non-vaccinated animals.

Conclusion: a RecA-mutant and a PurD-mutant of *Ornithobacterium rhinotracheale*, if given as such, are not capable to raise a protective immune response in chickens.

Please amend page 20, lines 26-28:

As follows from **Table 5**, concurrent aerosol vaccination of 1-day-old SPF broilers with live attenuated NDV and *recA* or *purD* mutant strains appeared safe and to induce a good level of immunity against challenge with wild type *Ornithobacterium rhinotracheale*.

Please amend page 21, lines 1-10:

In this experiment, Infectious Bronchitisvirus type MA5 was used as the live attenuated viral component. Additionally, the experiment with *Ornithobacterium rhinotracheale*/NDV combination vaccines was repeated.

The experimental set-up of this Example was largely identical to that of Example 5. Where different numbers of animals or different moments of vaccination or challenge apply, this is indicated in **Table 6**. This table also gives an overview of the vaccines used and the vaccination schedule, as well as the level of protection obtained.

MA5 suspensions: live attenuated Infectious Bronchitisvirus (IBV) type MA5 (Intervet International B.V., Wim de Korverstraat 35, 5831 AN Boxmeer, The Netherlands) was used at a concentration of  $5.5 \log^{10}$  EID<sub>50</sub> per animal, and applied by spraying.

Please amend page 21, lines 12-18:

### **Conclusion**

As follows from **Table 6**, concurrent aerosol vaccination of 1-day-old SPF broilers with IBV MA5 and PurD-mutant strains induces a good level of immunity against challenge with wild-type *Ornithobacterium rhinotracheale*. Additionally it follows from **Table 6**, that concurrent aerosol vaccination of 1-day-old SPF broilers with NCD and PurD-mutant strains induces a good level of immunity against challenge with wild-type *Ornithobacterium rhinotracheale* as was also demonstrated in Example 5.

Please amend page 22, line 7-10:

Figure 2A: Sequence of *purD* and localization of primers used for cloning and verification of mutants.  
**[SEQ ID NO.: 9]**

Figure 2B. Sequence of *recA* and localization of primers used for cloning and verification of mutants .  
**[SEQ ID NO.: 10]**

On page 22, following line 10, please insert:

**Table 5: Detailed experimental design and treatment schedule for the challenge experiment described in Example 5.**

**Table 6: Detailed experimental design and treatment schedule for the challenge experiment described in Example 6.**

***Amendments to the Figures***

Following Figure 2A, insert **[SEQ ID NO.:9]** .s

Following Figure 2B, insert **[SEQ ID NO.:10]** .